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REPORT ON STANDARDIZATION OF MICROCHEMICAL METHODS

MICRO KJELDAHL NITROGEN DETERMINATION

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The 1949 collaborative studies on the standardization of microchemical methods were limited to determination of nitrogen by the Kjeldahl procedure, since it was necessary to establish an official method for inclusion in the seventh edition of the Association's *Book of Methods*. The 1948 studies proved that the micro Kjeldahl method (tentative) was unsatisfactory for refractory compounds, such as nicotinic acid, which contain ring nitrogen. However, these studies indicated that a satisfactory Kjeldahl method might be evolved for the analysis of these compounds, since several of the 1948 collaborators using different methods obtained nitrogen values for nicotinic acid in agreement with the theoretical value. Willits, Coe, and Ogg¹ reported a satisfactory procedure for macro samples, and later work at the Eastern Regional Research Laboratory indicated that the same procedure was adaptable to micro samples. In this adaptation, the authors stressed the importance of (a) the concentration of potassium sulfate in the digestion mixture, (b) the time of digestion, and (c) the temperature of the digestion mixture. This micro digestion mixture contained the same ratios of potassium sulfate and of mercuric oxide to sulfuric acid as that recommended by Willits *et al.* The minimum time of digestion was critical, requiring at least 3 hours. It is perhaps this latter condition, together with the temperature of the digest, that was largely responsible for the poor results obtained by the 1948 collaborators, who reported 36 values out of a total 75 which ranged from 0.25 to 9.75 per cent as compared with the theoretical value of 11.38 per cent. The method used in the 1949 collaborative study for the analysis of nitrogenous compounds other than those containing N-N, NO, and NO₂ recommended a digestion mixture containing a larger amount of potassium sulfate, with more exact limits for each milliliter of sulfuric acid. The amount of sulfuric acid to be used was based on the amount of organic matter to be digested. The collaborators were instructed to digest the sample for 4 hours, with the digestion mixture boiling vigorously enough to cause the acid to reflux halfway up the neck of the flask. The two samples submitted for analysis—nicotinic acid and tryptophane—are both refractory compounds.

Sixteen collaborators participated, reporting 87 analyses for nicotinic

* One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

¹ Willits, C. O., Coe, M. R., and Ogg, C. L., *This Journal*, 32, 118 (1949).

acid and 74 for tryptophane. These analyses are shown as histograms in Figures 1 and 2.

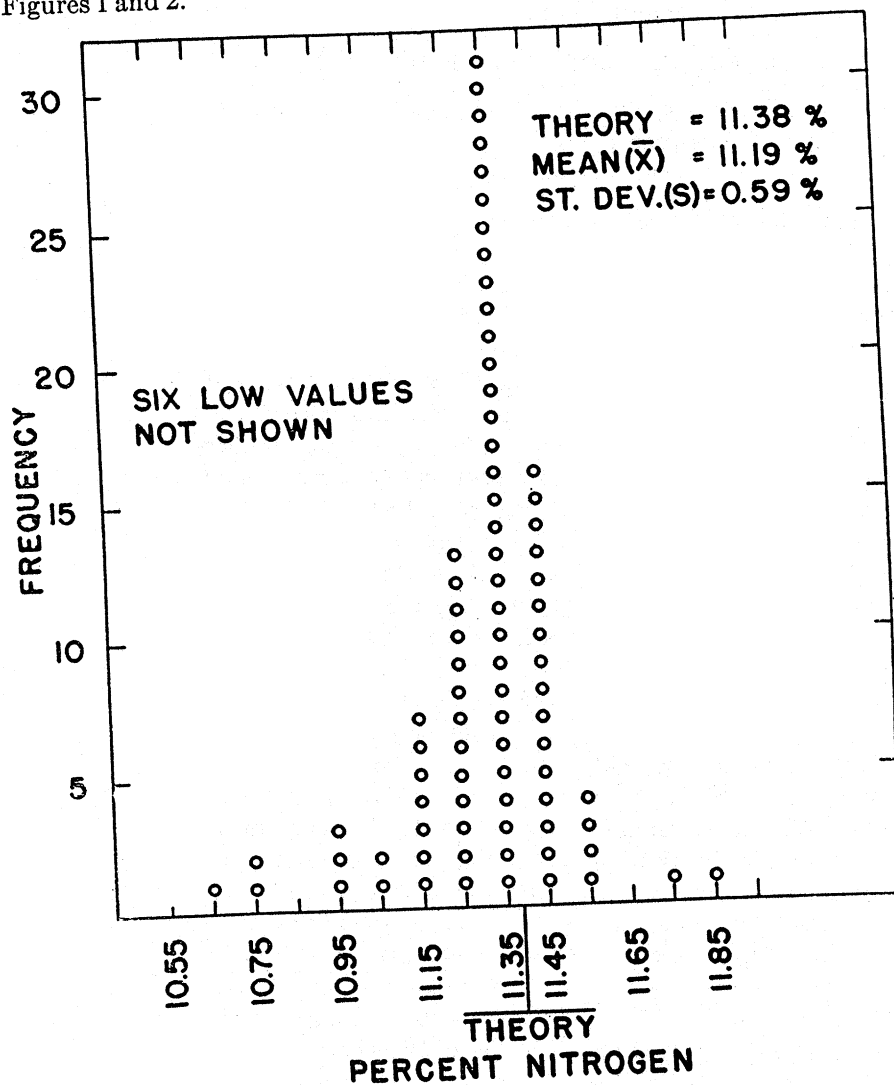


FIG. 1.—Kjeldahl nitrogen values for nicotinic acid.

The 87 nicotinic acid analyses ranged from a low of 9.16 per cent to a high of 11.86 per cent with a mean of 11.19, as compared with the theoretical value of 11.38 per cent. The chi square test, to determine whether or not these analyses represented a normal population, showed that 11 values were either too high or too low. The remaining 76 values, ranging from 10.96 to 11.56 per cent, had a standard deviation of 0.103 per cent

from a mean of 11.33 per cent, which is only 0.05 per cent below the theoretical value. Thus, 83 per cent of the total number of analyses had a standard deviation of only 0.103 per cent.

The 74 tryptophane nitrogen analyses gave values between 12.26 and 14.55 per cent, with a mean of 13.65 per cent, which when compared with

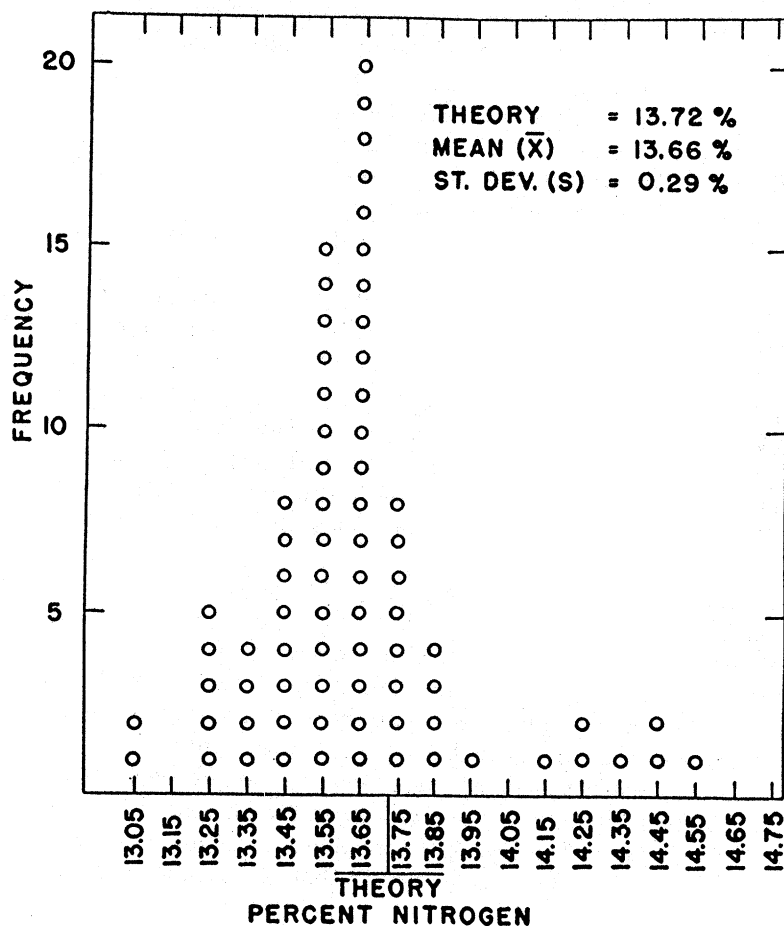


FIG. 2.—Kjeldahl nitrogen values for tryptophane.

the theoretical value of 13.72 per cent deviates by only 0.07 per cent. Of these 74 values, 3 were rejected by the chi square test. The remaining 71 values, constituting the normal population, ranged from 13.03 to 14.35 per cent, with a mean of 13.61 per cent, only 0.11 per cent below the theoretical value. Thus 96 per cent of the 74 tryptophane analyses had a standard deviation of 0.238 per cent.

In addition to the nitrogen analyses of these two compounds, each

collaborator reported the nitrogen obtained by the prescribed procedure for the particular compounds used by them as test or standard materials. There were 79 of these analyses of test materials, including 36 for acetanilide and 16 for cystine; the other 27 were distributed between benzyliso-thiourea, phenyl-thiourea, ammonium sulfate, nicotinic acid, glutamic acid, and tryptophane. Although these analyses were not done collaboratively, that is, each analyst's material was not an aliquot of the same lot, they were compounds of relatively well established purity. To permit comparison of the nitrogen data from these different compounds, it was necessary to treat the data in a somewhat different manner and to assume that these compounds were all pure. Thus, the values used represented the differences between that found and the theoretical values for nitrogen regardless of the percentage of the contained nitrogen, and they were treated either as an individual compound or as if all values were of a single compound.

The 36 Kjeldahl analyses of acetanilide gave nitrogen values which represented a normal population with a standard deviation of only 0.137 per cent. The values ranged from a low of 10.04 to a high of 10.52 per cent, with a mean of 10.27 per cent, which is only 0.09 per cent lower than the theoretical value of 10.36 per cent for nitrogen.

The 16 analyses of the standard compound cystine also yielded values representing a normal population with a small standard deviation of 0.078 per cent. The mean of 11.66 per cent is identical with the theoretical value.

The standard deviation for the entire 79 analyses, which included all the various standard compounds, was 0.127 per cent, and the mean of the differences from the theoretical value was -0.056 per cent.

These data indicate that the Kjeldahl method used in the 1949 collaborative studies can be applied successfully to both easily decomposed and refractory nitrogenous materials for the analysis of nitrogen.

The probability is that more than 95 per cent of all the results obtained by this method will fall within a normal population and that the standard deviation will be 0.17 per cent, the *s* value for all the analyses reported.

The Referees therefore recommend* that the following procedure be accepted as first action.

MICRO-KJELDAHL PROCEDURE

(not applicable to compounds with N-N, NO, or NO₂ linkages).

REAGENTS

(a) Indicator:

1. Methyl red-methylene blue. Mix 2 parts of 0.2 per cent methyl red with 1 part of 0.2 per cent methylene blue, both in 95 per cent ethanol.
- or 2. Methyl red-bromocresol green. Mix 5 parts of 0.2 per cent bromocresol green with 1 part of 0.2 per cent methyl red, both in 95 per cent ethanol.

* For report of Subcommittee C and action of the Association, see *This Journal*, 33, 54 (1950).

- (b) *Sodium hydroxide-sodium thiosulfate soln.*—Aqueous soln of 50 g of NaOH and 5 g of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ /100 ml.
- (c) *Boric acid soln.*—Dissolve 4 g of H_3BO_3 per 100 ml. of distilled water.

DETERMINATION

Weigh a 10- to 30-mg sample* on a micro or semimicro balance and transfer to

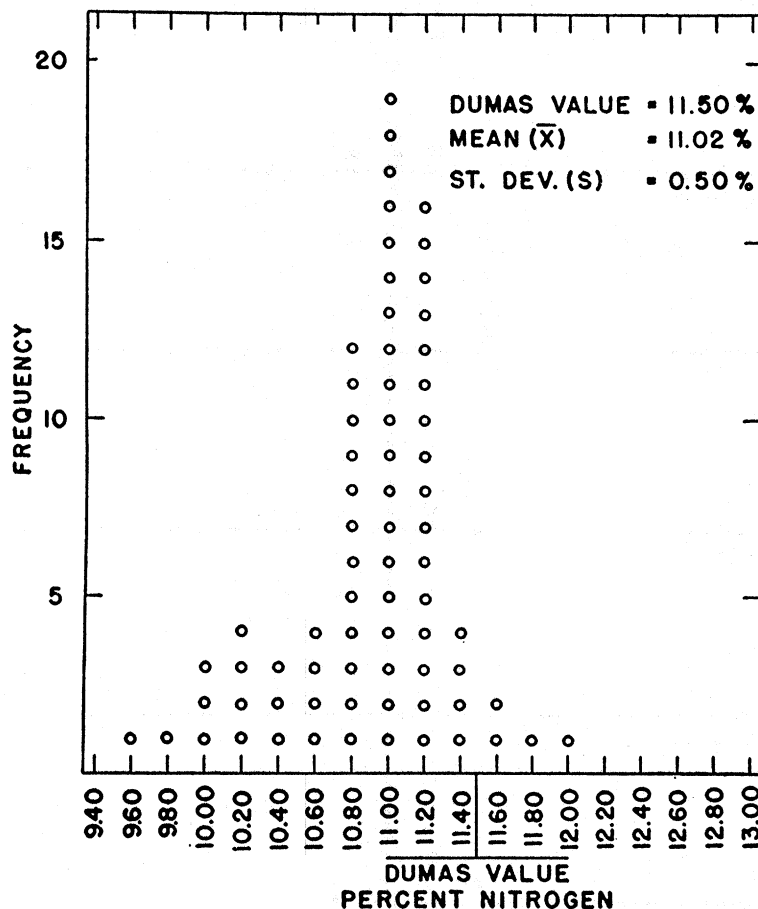


FIG. 3.—Kjeldahl nitrogen values for methyl orange.

a 30-ml Kjeldahl digestion flask. Weigh the sample either in a charging tube or on a piece of cigarette paper. Add to the sample 1.30 ± 0.05 g. potassium sulfate, 40 ± 5 mg mercuric oxide and 2.0 ml concentrated sulfuric acid. Add boiling chips and digest for 4 hours (digest for 1 hour if sample is known to contain only amines, amides, or other easily digestible materials) by boiling *vigorously*, with the acid condensing well up into the neck of the flask. Cool, add a minimum of distilled water (about 5 ml) to dissolve the solids, and place a thin film of vaseline on the lip of the flask.

* For samples of less than 10 mg, weigh on a micro balance and use one-half the specified amounts of the reagents except where the total organic matter in the digest (sample + cigarette paper, if used) exceeds 10 mg. Collect the distillate in a 50 ml Erlenmeyer flask, dilute to 25 ml and titrate with 0.01 N HCl.

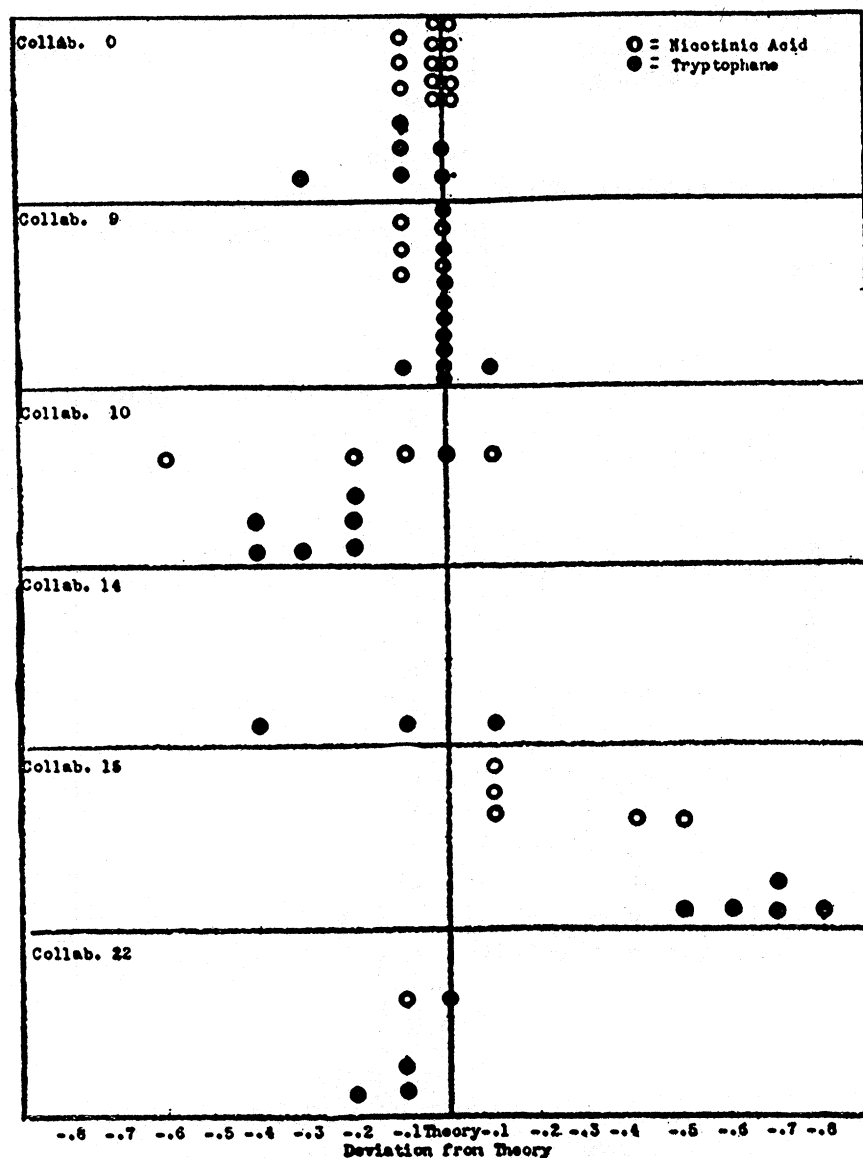


FIG. 4.—Distribution of Kjeldahl values. (Method I)

Transfer the digest including the boiling chips to a micro or semimicro steam distillation apparatus of standard design. To condition the apparatus after periods of disuse, pass steam through it for several minutes. Completeness of transfer of the digestion mixture can be tested by the addition of a drop of methyl orange indicator to the final rinse. Place the 125-ml Erlenmeyer receiver containing 5 ml of 4% boric acid and 4 drops of the indicator under the condenser with the tip extending below the surface. Add 8 ml of the sodium hydroxide-sodium thiosulfate reagent to the still, and steam distill until ca 15 ml of distillate is collected. Dilute the contents of

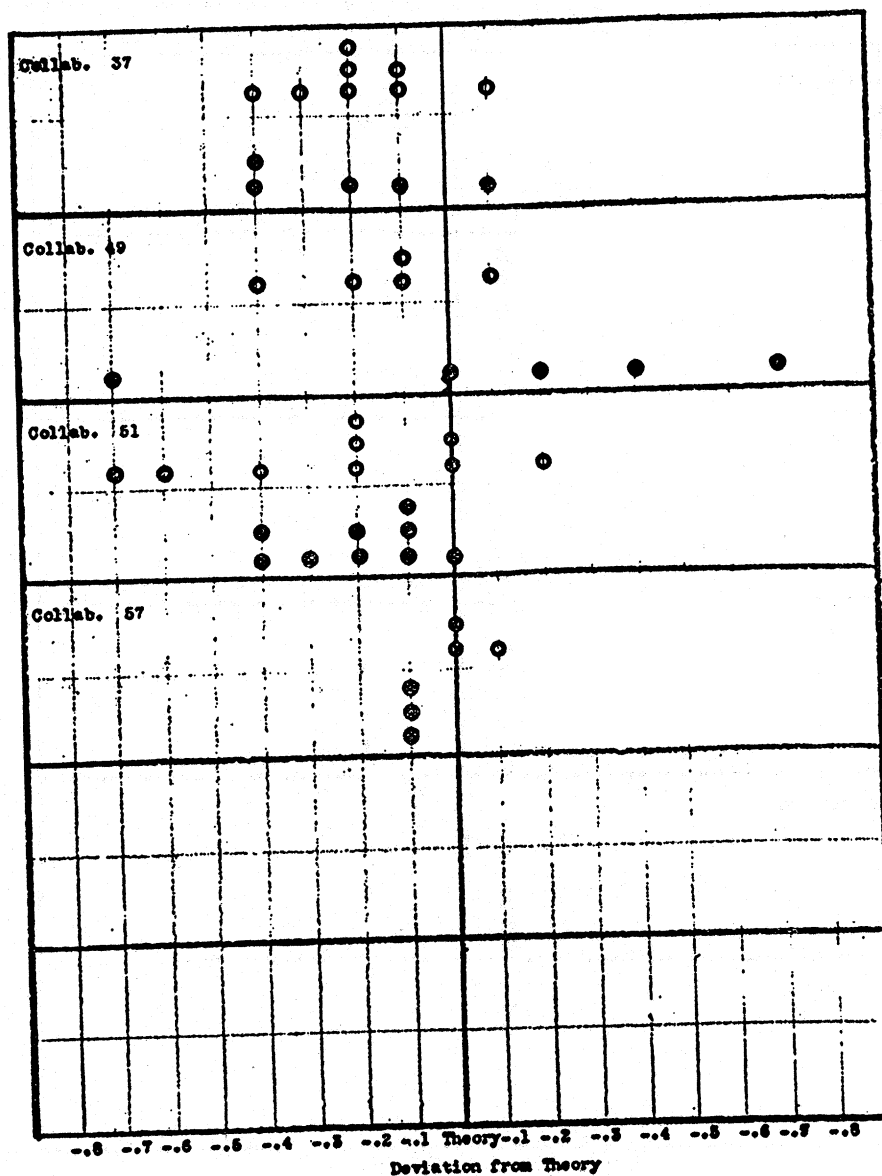


FIG. 4. (contd.)—Distribution of Kjeldahl values. (Method I)

MICRO KJELDAHL ANALYSIS OF COMPOUNDS CONTAINING N-N, NO, OR NO₂ LINKAGES

The collaborators were also requested to analyze a sample of methyl orange so that the tentative Friedrich method for nitrogen by the micro Kjeldahl method (for N-N, NO, and NO₂ linkages) could be appraised.

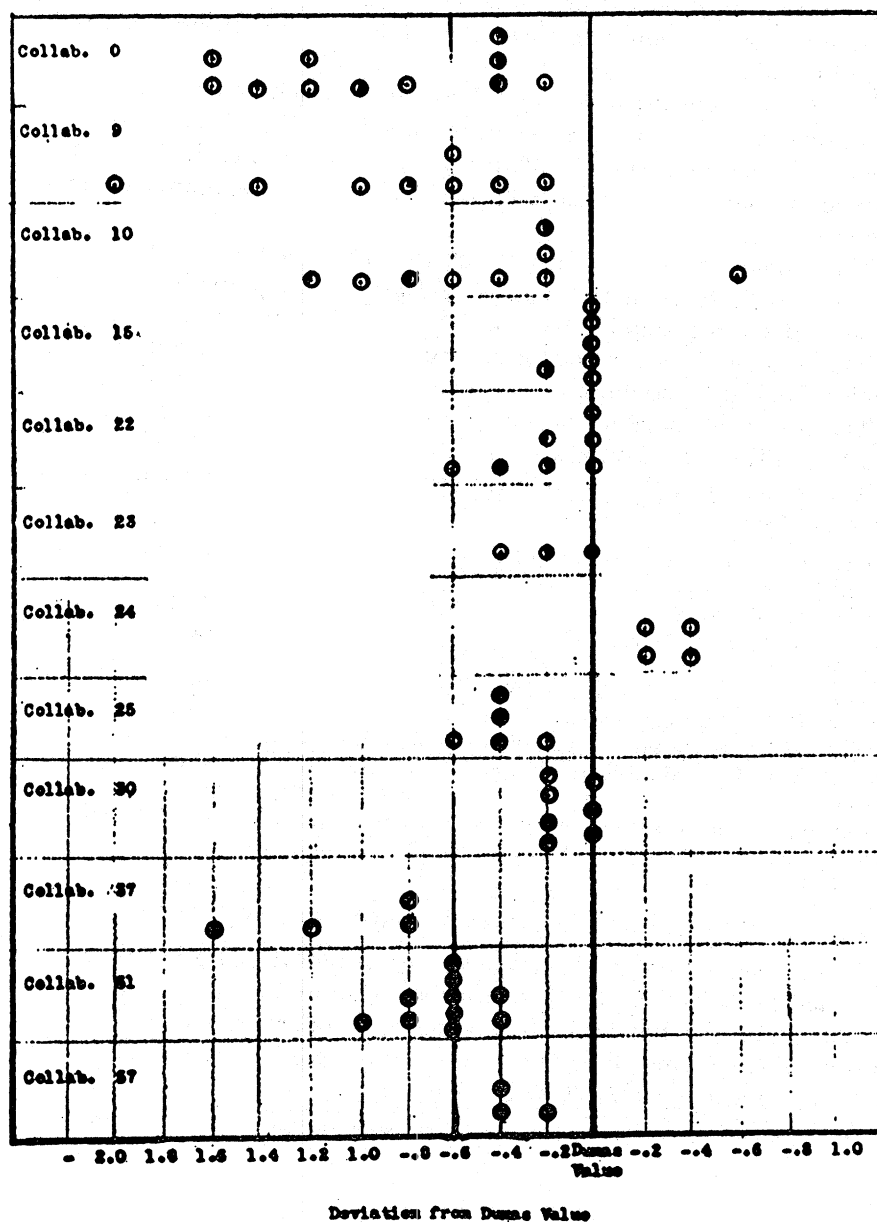


FIG. 5.—Distribution of Kjeldahl values for methyl orange. (Method II)

Seventy-nine micro Kjeldahl analyses were reported, ranging from a low of 9.57 to a high of 12.11 per cent, with a mean of 11.02 per cent. The analyses are shown in Figure 3.

Because the compound was known to be impure, this mean was com-

pared with a nitrogen value of 11.50 per cent obtained as an average of 10 analyses by the Dumas method rather than with the theoretical value of 12.84 per cent.

The standard deviation of these values, 0.496 per cent, is extremely high indicating poor precision. The confidence limits, ± 0.106 per cent ($2s/\sqrt{n}$), when added to the mean of 11.02 per cent, did not approach the Dumas nitrogen value; therefore, this method must be considered unreliable and unacceptable.

The Referees therefore recommend* that the Friedrich method (tentative) be deleted from the *Book of Methods* and that further work be done to develop a suitable method which can be recommended as first action for analysis of compounds having an N-N linkage for nitrogen by a micro Kjeldahl procedure.

The analytical values submitted by the various collaborators for nitrogen by the Kjeldahl method are presented graphically in Figures 4 and 5.

The following is the list of those collaborators who participated in these studies:

Brown, L. E., Southern Regional Research Laboratory	Ketchum, D. E., Eastman Kodak Company
Brunner, A. H., Ansco	Miller, L., University of Michigan
Butler, A. Q., Mallinkrodt Chemical Works	Ogg, C. L., Eastern Regional Research Laboratory
Deering, A. W., Hunter College, N. Y.	Steyermark, A., Hoffman-LaRoche, Inc.
Dutton, C. D., Picatinny Arsenal	Sundberg, O. E., Calco Chemical Div., American Cyanamid Co.
Grodsky, J., Ortho Research Foundation	Van Etten, C. H., Northern Regional Research Laboratory
Hallett, L. T., General Aniline and Film Corp.	White, L. M., Western Regional Research Laboratory
Hageman, B., The Texas Company	
Jones, G. A., E. I. DuPont de Nemours and Co., Inc.	

METHOXYL AND ETHOXYL GROUPS—TENTATIVE

The procedure for the determination of methoxyl and ethoxyl groups 41.1 and 41.2, 6th Ed., has been used successfully in the Associate Referee's and other laboratories. The Referee, therefore, recommends* that this method be adopted as official. The procedure has been reedited for publication in 7th Ed. *Methods of Analysis*.

* For report of Subcommittee C and action of the Association, see *This Journal*, 33, 54 (1950).